Effect of salt stress on activity of superoxide dismutase (SOD) in *Ulmus pumila* L.

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Abstracts: The injury tolerance of cell plasma membrane and the correlative enzymes activities of plasma-membrane protection system in the *Ulmus pumila* leaves treated by nine concentrations (0.3%, 0.6%, 0.9%, 1.2%, 1.5%, 1.8%, 2.1%, 2.4%, 3.0%) of Na_2CO_3 and $NaHCO_3$ mixtures were studied in a greenhouse of Northeast Forestry University, Harbin, China. The rate of electrolyte leakage (REL) and SOD (Superoxide dismutase) activity in leaves of different samples were determined. Results showed that the REL in leaves of *U. pumila* presented a slowly increasing trend at the salt concentrations less than 1.5%, which indicated that cell plasma membrane of *U. pumila* leaves had rather strong resistance to the injury of salt ion, and had a significant increase at the salt concentrations more than 1.5%. The SOD activities in leaves of *U. pumila* presented an increased trend at salt concentrations less than 1.5%, the growth of seedlings did not decline, and tress and leaves had no symptom of injury, while the salt concentrations exceeded 1.5%, SOD activities sharply decreased and REL increased promptly.

Keywords: *Ulmus pumila*; Salt stress; Rate of electrolyte leakage (REL); SOD

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Introduction

Salt tolerance of plants mainly depended on the stability of cell membrane system which can still keep the integrality under salt stress to maintain the selective absorption for ion and other physiological function of cell (Greenway & Munne 1980). Salinity induced a wide range of responses in plants, and severe salt stress may cause oxidative damages, ion toxicity, nutritious imbalance, and plant death (Xiong *et al*. 2002). During normal metabolism, plants generated reactive oxygen species (ROS), including superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (HO^-), and singlet oxygen (O_2^1) (Zhang *et al*. 2005), and the generation and elimination of ROS kept a dynamic balance at all times. When plants were stressed by bad environment, this balance was broken and ROS was overproduced in plants. Meantime, the biosynthesis of some antioxidant enzymes (SOD, POD, PPO, and MDA, CAT, and so on) in plants was induced by superabundant ROS, which can repair the DNA injury in time to maintain the normal growth of plants. Many microorganisms and plants also had the function inducing antioxidant defense system under environmental stresses (Shi *et al*. 2004; Ireneusz *et al*. 2003). ROS induced by salt stress played important roles in adaptive responses at lower concentrations, while it caused damages to macromolecules, such as DNA, proteins, membrane lipids, at higher concentrations (Wang *et al*. 2004; Mittler 2002; Breusegem *et al*. 2001). Injury caused by ROS is known as oxidative stress, which is the major cause of damage in plants exposed to different stressors (Bowler *et al*. 1992). Therefore much attention has been paid in recent years to the capacity that balanced the generation and elimination of ROS in plant cells under environmental stresses (Mittler 2002). Plants have well-developed defense systems against ROS, but the mechanisms are not well understood (Alscher *et al*. 2002). Superoxide dismutase (SOD) is one of the crucial enzymes that protect cells against the oxidative damages (Raychqudhuri & Deng 2000; Fridovich 1986), which is the key enzyme to diminish the concentrations of ROS (Slooten *et al*. 1998). SOD catalyzes the redundant superoxide radicals (O_2^-) to yield molecular oxygen and hydrogen peroxide (H_2O_2). The control of the steady-state O_2^- levels by SOD is an important protective mechanism against cellular oxidative damage, since O_2^- acts as a precursor of more cytotoxic or highly reactive oxygen derivatives, such as peroxynitrite or HO^- (Halliwell & Gutteridge 1999). Therefore, SOD is usually considered the first line of defense against oxidative stress (Sigaud-Kutner 2002). Increased SOD activity was correlated with increased protection from damage associated with oxidative stress (Asada 1999).

Ulmus pumila L. (Ulmaceae), a widespread tree species in North China, with good performance of fast growth, high quality, strong adaptability and resistance, and high economy value, has been used as a major forestation species of shelter forest and saline alkali land in recent years, especially in the semi-arid sand land (Shi 2004; Yan *et al*. 1997). In recent years, some studies have been conducted on this species, but these studies mostly focused on the provenance trails (Wu *et al.* 2001), heredity improvement and variation (Gu *et al*. 1987; Ma *et al*. 1990; Song *et al*. 1995; Sun *et al.* 1999), gas exchange, biomass allocation, preventions of insect pest and disease (Solla *et al*. 2005; Wang and Wang 2005; Qu *et al*. 1999), raising seedling techniques (Zhang *et al*. 2003) of *U. pumila*, and so on. However, little attention has been paid to the permeability and relative antioxidant enzymes activities of protection system in cell plasma membrane for *U. pumila* exposed to salt stress. The roles of SOD under environmental stresses have been studied extensively (Raychqudhuri & Deng 2000; Yu & Rengel 1999), but little is known about the change of SOD activities in *U. pumila* under salt stress. Therefore, in order to clarify the tolerant mechanism of antioxidant enzymes against salt stress, we describe the osmo-

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sis of plasma membrane system and activities of SOD in *U. pumila* leaves exposed to salt stress in this study. This work will provide help for better understanding the roles of SOD in adaptive responses of plant cells under environmental stresses.

Materials and methods

Plant material and stress conditions

One-year-old *U. pumila* seedlings were provided by Fengle Nursery of Zhaozhou County, Heilongjiang Province of China. The samples were transplanted into pots containing riversand pretreated by tap water in greenhouse of Forest Tree Breeding Base of Northeast Forestry University. The seedlings were fertilized complex fertilizer and watered termly to ensure the natural growth of seedlings before salt treatments. Subsequently, the seedlings were treated for seven days by two alkaline salts ($NaHCO_3$ and Na_2CO_3) selected based on the salt components in the extant salt-alkaline soil of northeast China (Shi and Wang 2005). The mixture of Na_2CO_3 and $NaHCO_3$ had nine concentrations of 0.3%, 0.6%, 0.9%, 1.2%, 1.5%, 1.8%, 2.1%, 2.4%, 3.0%, and the control. All of treatments were repeated at least five times.

Determination of membrane permeability

Membrane permeability can be reflected by the rate of electrolyte leakage (REL). In our study it was determined as described by Lutts *et al*. (1996). One gram of fresh living leaf was taken from each pot and cut into 1-cm long segments, then washed three times with deionized water to remove surface-adhered electrolytes. The leaf segments were divided equally and placed into two closed vials, each containing 20 mL of deionized water. One vial was incubated at 25°C on a rotary shaker for 3 h, and then the electrical conductivity of the solution (EC1*)* was determined with a conductivity gauge. The other vial was autoclaved at 120°C for 20 min and electrical conductivity of the solution (EC2*)* was determined after equilibration to 25°C. REL can be defined as follows:

$$REL\ (\%) = (EC1/EC2) \times 100.$$

SOD activity assay

Ten grams of frozen leaf samples was homogenized on ice with a mortar and pestle for 2 min in 10 mL of homogenizing solution containing 50 $mmol \cdot L^{-1}$ HEPES buffer and 0.1 $mmol \cdot L^{-1}$ Na_2EDTA (pH 7.6). The homogenate was centrifuged at 10 000 $r \cdot m^{-1}$ for 20 min at 4 °C and the supernatant was used for SOD assays (Yu and Rengel 1999).

SOD activity was assayed by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium (NBT), according to the method of Giannopolitis and Ries (1977a, b) with some modifications. For the total SOD assay, a 5-mL reaction mixture, which contains 50 mm HEPES (pH 7.6), 0.1 $mmol \cdot L^{-1}$ EDTA, 50 $mmol \cdot L^{-1}$ Na_2CO_2 (pH 10.4), 13 $mmol \cdot L^{-1}$ methionine, 0.025% (w/v) Triton X-100, 75 $\mu mol \cdot L^{-1}$ NBT, 2 $\mu mol \cdot L^{-1}$ riboflavin and an appropriate aliquot of enzyme extract, was illuminated for 10 min at a light intensity of 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$. A control reaction was always performed wherein all the steps and components were exactly the same as described above, except that crude enzyme was replaced with an equal volume of phosphate buffer (pH 7.8) (Sahoo *et al*. 2001). One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm.

Results and analysis

Change of plasma membrane under salt stress

The permeability of plasma membrane was one of the major physiological indexes of osmosis-resistant stress in plants, which reflected directly the stabilization ability of cell for its internal environment and the adaptive and resistant abilities for external environmental change. The effects of salt on structure and constitute of plasma membrane were important aspects of salt stress for plant toxicity, and under high concentration of salt stress, membrane systems of plants were exposed to damage firstly (Zhang *et al*. 2004). With the increase of external salt concentration, the function of cell membrane may be changed by salt ion stress, and intracellular electrolyte began to leak. From Fig. 1, we can see that the rate of electrolyte leakage (REL) changes of *U. pumila* leaves treated presented an increased trend with the increase of salt concentration. At the treatments of salt concentrations less than 1.5%, the REL of samples increased slowly. This phenomenon indicated that cell plasma membrane of *U. pumila* leaves had rather strong resistance to the toxicities of salt ions, and the occurrence of injuries was relative slow. Thus, we can conclude that there exists a half lethal concentration for the injury of cell plasma membrane at the salt concentration less than 1.5%. Whereas, at the salt concentration more than 1.5%, the REL of samples sharply increased, and intracellular electrolyte began to largely leak, which revealed that cell plasma membrane of sample treated had suffered from the severe injury.

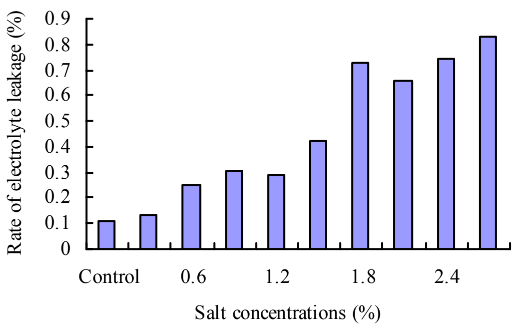

Fig.1 The rate of electrolyte leakage of *U. pumila* leaves at the different salt concentrations

Effect of salt stress on activity of SOD

The activities of SOD were determined in *U. pumila* leaves treated by nine concentrations of salt mixtures. The results showed that SOD of *U. pumila* leaves was induced at salt stress, and the activities of SOD had obvious change (Fig. 2). At the treatments of salt concentrations less than 1.5%, SOD activities presented an increased trend, the growth of seedlings did not decline, and tress and leaves had no symptom of injury. These phenomena showed that the antioxidative defense system in *U. pumila* leaves was not damaged rapidly and can be repaired in a short time under low salt concentrations. While the salt concentrations exceeded 1.5%, SOD activities sharply decreased and REL increased promptly. This indicated that the accumulation of ROS in *U. pumila* leaves had exceeded the regulating thresholds by itself under these conditions. The overproduction of ROS in plant can not be scavenged, which led to decrease or decomposition of SOD activities. Thus, further analyses showed that al-

though plants may enhance their tolerances for salt stress by increasing the activities of SOD to a certain extent, the ROS may still cause the oxidative damage to plasma membrane and inhibits its plant growth when SOD in plants can not scavenge all ROS generated by salt stress.

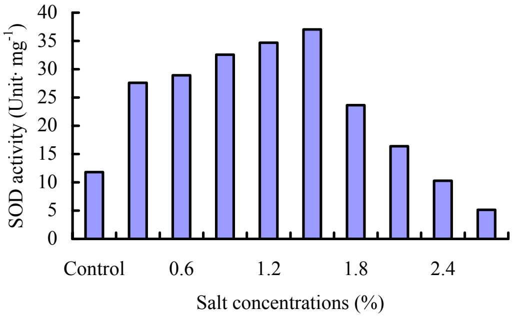

Fig.2 Activities of SOD in *U. pumila* leaves at the different salt concentrations

Discussion

The injuries of osmosis stresses for plants, such as drought, salinity, and chilling, etc., has close relation with oxidative stresses of cell level, and the oxidative stress resistance is also an important measure of salt tolerance for plants. Increased permeability of plasma membrane under salt stress is an expression of plant organ suffering from toxicity (Hasegawa *et al*. 2000). Our study result showed that at the treatments of salt concentrations more than 0.6%, the REL of *U. pumila* leaves also had an increase compared with that of the control. Lower salt concentrations had a slight injury for plant cells, and with salt concentrations increasing, the structure and function of cell plasma membrane were damaged, moreover, various intermetabolic pathways of plants were inhibited, and growth clearly declines (Zhang *et al*. 2003; Xia *et al*. 2005).

The overproduction of intracellular reactive oxygen species (ROS) may result in peroxidation of plasma membrane and oxidative damage (Lin 2004). As a mainly protective mechanism of eliminating ROS in organism, antioxidant enzyme system may enhance the biosynthesis abilities of antioxidant enzymes with the increase of ROS. Thus, the changes of activities of antioxidant enzymes reflects indirectly the existence of harmful substances in circumstance, and it is one of sensitive biological markers in forecasting the toxicities of environmental stresses for ecosystem at molecular levels (Banerjee *et al*. 1999; Li *et al*. 2002). Much evidence obtained from various plant systems showed that the amounts and activities of enzymes involved in scavenging ROS were changed by environmental stresses (Madamanchi *et al*. 1994). Leguminous plants exposed to salt stress showed that the ROS, including O_2^- and H_2O_2, were generated largely. Though O_2^- and H_2O_2 had a little toxicity for plant, they could be transformed to high toxicity of OH^- through Habre-Weiss reaction. This ROS may damage intracellular biomacromolecule, resulting in DNA injury, enzyme deactivation, and peroxidization of membrane lipid, affecting synthesis and stability of protein, and causing function disorder and cell death (Li *et al*. 2002; Bai and Wang 2002; Pan *et al*. 2003). As an important antioxidant enzyme scavenging ROS in vivo, the induction of SOD activity has been reported by many literatures for plant subjected to environmental stresses (Madamanchi *et al*. 1994; Chen *et al*. 1999; Hermandwz *et al*. 2002). In our experiment, the induction action of low salt concentrations for SOD activities of *U. pumila* leaves was an adaptive reaction of plant for salt stress to enhance the ability of eliminating ROS. While the inhibition of high salt concentrations for SOD activities can be thought that the tolerance of plant for salt stress has exceeded itself adaptability, and the decrease of SOD activity caused by stress may be regarded as an omen of plant toxicosis (Ireneusz *et al*. 2003; Dong *et al*. 2001; Zhang *et al*. 2005). The results of this study indicated that increased SOD activities may enhance the ability of its scavenging ROS for *U. pumila* to salt stress, and the plant may grow normally. The activities of SOD and the resistance of plant had a certain correlation.

Conclusion

The rate of electrolyte leakage (REL) changes in *U. pumila* leaves treated had an increased trend with the increase of salt concentration. At the treatments of low salt concentrations, the REL of samples increased slowly. This phenomenon indicated that cell plasma membrane of *U. pumila* leaves had rather strong resistance to the injury of salt ion, and the injury occurred relatively slowly. Whereas, at the salt concentration more than 1.5%, the REL of samples sharply increased, and intracellular electrolyte began to largely leak, which showed cell plasma membrane of samples treated had suffered from the severe injury.

The amounts and activities of SOD in *U. pumila* leaves had high levels under salt stress. SOD can make a rapid reaction for environmental stresses, with high abilities in scavenging reaction oxygen species and keeping the balance of reactive oxygen metabolism, and can effectively avoid the oxidative damages of reactive oxygen species for plants under a certain concentration of salt stress.

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